1.15925.0025

Microscopy

Orange G Certistain® C.I. 16230

IVD In Vitro Diagnostic Medical Device CE

Application

Dye for Papanicolaou's staining in cytology.

The most used staining procedure for cytological specimen is Papanicolaou's technique. In the first staining step the nuclei are stained by a hematoxylin solution. Nuclei are stained blue, dark violet to black.
The second staining step is cytoplasmic staining by orange staining solution, especially for demonstration of mature and keratinized cells. The target structures are stained orange in different intensities. In the third staining step the so-called polychromatic solution is used, a mixture of eosin, light green SF and Bismarck brown. The polychromatic solution is used for demonstration of the differentiation of squamous cells.

Sample material

Gynaecological and non-gynaecological specimen as sputum, urine, FNAB, body effusions, lavages

Fixation

Wet fixation immediately with spray fixative Merckofix® or wet fixation immediately in 96% ethanol for min. 30 min

Reagents

Cat. No. 115925	Orange G Certistain®	25 g
Colour Index No.	C.I. 16230	
Colour Index Name:	Acid Orange 10	
Cat. No. 100583	Tungstophosphoric acid hydrate GR	100 g
Cat. No. 100971	Ethanol 96% pure	1 l

Additional necessary

Cat. No. 109253	Papanicolaou's solution 1a, Harris hematoxylin solution	500 ml, 1 l, 2.5 l
Cat. No. 109254	Papanicolaou's solution 1 b, Hematoxylin solution S	500 ml, 2.5 l
Cat. No. 109271	Papanicolaou's solution 3a, polychromatic solution EA 31	500 ml, 2.5 l
Cat. No. 109272	Papanicolaou's solution 3b, polychromatic solution EA 50	500 ml, 1 l, 2.5 l
Cat. No. 109270	Papanicolaou's solution 3c, polychromatic solution EA 65	100 ml
Cat. No. 109269	Papanicolaou's solution 3d, polychromatic solution EA 65	100 ml, 2.5 l

Preparation

Orange G solution

Dissolve 0.5g Orange G Certistain® in 100 ml ethanol, add 0.015 g tungstenphosphoric acid and stir until it is dissolved. Filter before use.

Procedure

Conventional staining, manual:

1. Wash with 96 % alcohol*.
2. Wash with 80 % alcohol*.
3. Wash with 70 % alcohol*.
4. Wash with 50 % alcohol*.
   *If Merckofix® is used, steps 1 – 4 can be dropped
5. Wash with distilled water
6. Stain in Harris' hematoxylin solution 3 min or hematoxylin S solution 2-3 min
7. Rinse under weak stream of tap water 3-5 min
8. Wash with 50 % alcohol
9. Wash with 70 % alcohol
10. Wash with 80 % alcohol
11. Wash with 96 % alcohol.
12. Stain in orange G solution for 3 min
13. Wash with 96 % alcohol
14. Wash with 96 % alcohol
15. Stain in polychromatic staining solution EA 31or EA 50 for 3 min
16. Dehydrate with 96 % alcohol
17. Dehydrate with 96 % alcohol
18. Dehydrate with absolute alcohol 5 min
19. Dehydrate with equal parts of absolute alcohol and Neo-Clear® or xylene
20. Clear with Neo-Clear® or xylene
21. Clear with Neo-Clear® or xylene for 2 min

Mount with Neo-Mount® (for Neo-Clear®) or Entellan®new (for xylene).

Result

Staining with	**3a/EA 31**	**3b/EA 50**	**3c/EA 65**	**3d/EA 65**
Cytoplasm				
Cyanophilic (basophilic)	intense green	blue-green	light red	pale blue-green
Eosinophilic (acidophil)	pink	pink	red	pink
keratinized	pink-orange	pink-orange	brownish-red	pink-orange
Erythrocytes	red	red	brownish-red	red
Nuclei	blue, black, dark lviolet			
Mikroorganisms	grey-blue			
Trichomonades	grey-green			

Technical note

The microscope used should meet the requirements of a medical diagnostic laboratory. Follow the instructions for the fixative.
The Papanicolaous staining solutions are ready to use, dilution is not necessary, might reduce the staining result and the self life.
If a staining analyzer is used, follow the instructions of the instrument and software company.
Remove surplus immersion oil before filing.

Sample preparations

All samples should be treated using state-of-the-art technology.
All samples must be clearly labelled.
Suitable instruments must be used for taking samples and their preparation; manufacturer instructions for application /use must be followed.

Diagnostics

Diagnoses are only to be made by authorized and trained persons.
Valid nomenclatures must be used. Suitable controls should be conducted with each application in order to avoid an incorrect result.
Further tests must be selected and implemented according to recognized methods.

Storage

The dye must be stored at +5°C to +30°C.
The dye must be used by the expiry date stated.

Shelf-life

After the first opening of the bottle the contents can be used up to the expiry date when stored at +5°C to +30°C.
The bottles must be kept tightly closed at all times.

Instructions for use

In order to avoid errors, the staining process must be carried out by an expert.

Only for professional use
National guidelines for work safety and quality assurance must be followed.
Microscopes equipped according to the standard must be used.
If necessary use a centrifuge suitable for medical diagnostic laboratory.

Protection against infection

Effective measures to be taken to protect against infection in line with laboratory guidelines.

Instructions for disposal

Used solutions and solutions that are past their shelf-life must be disposed of as special waste in accordance with local guidelines. If you have more detailed questions regarding disposal, these can be forwarded by e-mail: retrologistik@merck.de. Within the EU the currently applicable regulations on the basis of Directive 67/548/EEC of the Council for harmonization of the legal and administrative regulations apply for the classification, packaging, and labelling of hazardous substances.

Auxiliaries

Cat. No.	103981	Merckofix®	100 ml
Cat. No.	104699	Immersion oil	100 ml, 500 ml
Cat. No.	115577	Immersion oil acc. to ISO 8036 modified	100 ml
Cat. No.	106965	Oil of cedar wood	100 ml, 500 ml
Cat. No.	107961	Entellan® new	100 ml, 500 ml
Cat. No.	109016	Neo-Mount®	500 ml
Cat. No.	109843	Neo-Clear® (xylene substitute)	5 l
Cat. No.	100974	Ethanol denatured with 1% methyl ethyl ketone for analysis EMSURE®	1l, 2.5l
Cat. No.	100983	Ethanol absolute for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1l, 2.5 l
Cat. No.	108681	Xylene for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1l, 2.5l

Safety classification

Cat. No. 115925
Please observe the hazard classification on the label and the information given in the safety data sheet.
The Merck safety data sheet is available on the Internet and on request.

Main components of the product

Cat. No. 115925
C.I. 16230
$C_{16}H_{10}N_2Na_2O_7S_2$
M = 452.37g/mol
Dye content >/= 80%

Status: February 2011

Merck KGaA, 64271 Darmstadt, Germany,
Tel. +49(0)6151 72-2440
www.merck-chemicals.com/microscopy

1.15941.0025

Microscopy

Light green SF yellowish (C.I. 42095) Certistain®

IVD In Vitro Diagnostic Medical Device CE

Principle
Dye for histological stainings.
Light green SF yellowish is used for routine staining methods in histology, e.g. the staining of collagen fibers and especially for Masson's trichrome staining acc. to Goldner. Light green SF yellowish is used for Papanicolaou's polychromatic staining in cytology and is widely used as a counterstain.

Sample material
Paraffin sections

Reagents
Cat. No. 115941	Light green SF yellowish (C.I. 42095) Certistain®	25 g, 100 g
Color Index No.	C.I. 42095	
Color Index Name	Acid green 5, Food green 2	

Cat. No. 115973 Weigert's iron hematoxylin kit	2 x 500 ml
Cat. No. 115927 Ponceau S (C.I. 27195) Certistain®	25 g
Cat. No. 105231 Acid fuchsin (C.I. 42685) Certistain®	25 g
Cat. No. 115925 Orange G (C.I. 16230) Certistain®	25 g
Cat. No. 100532 Molybdatophosphoric acid hydrate GR	25 g
Cat. No. 100063 Acetic acid (glacial) 100% GR	1 l

Preparation
1. Ponceau S / Acid fuchsin solution
Dissolve 0.2 g Ponceau S Certistain® and 0.1 g acid fuchsin Certistain® in 300 ml distilled water and 0.6 ml acetic acid (glacial) and filter.

2. Orange G solution
Dissolve 2 g orange G Certistain® and 5 g molybdatophosphoric acid in 100 ml distilled water and filter.

3. Weigert's iron hematoxylin solution
Mix Weigert's solution A and Weigert's solution B in a ratio 1:1.

4. Acetic acid solution 1%
Add 10 ml acetic acid (glacial) to 990 ml distilled water.

5. Light green SF solution
Dissolve 0.2 g light green SF yellowish in 100 ml water and 0.2 ml acetic acid (glacial) and filter.

Procedure
Trichrome staining

Step	Time
Deparaffinize the sections in the typical manner and rehydrate	
Rinse in distilled water	1 min
Stain in Weigert's iron hematoxylin solution	3 min
Differentiate in running tap water	3-5 min
Stain in Ponceau S/acid fuchsin solution	3 min
Rinse in 1% acetic acid solution	
Stain in orange G solution	25 sec
Rinse in 1% acetic acid solution	
Stain in light green SF solution	5 min
Rinse in 1% acetic acid solution Dehydrate and clear 2 x 96% ethanol 2 x 100 % ethanol Clear in xylene or Neo-Clear® and mount with Entellan® new or Neo-Mount®	 1 min each 1 min each

Result
Nuclei	dark blue, black-brown
Cytoplasm	red
Connective tissue	green
Erythrocytes	orange

Technical note
The microscope used should meet the requirements of a medical diagnostic laboratory. Follow the instructions for the fixative.
Follow the instructions for the histoprocessing and the instructions of the equipment and software manufacturer.
Weigert's iron hematoxylin working solution remains stable for at least 5 working days if kept at 15-25°C. The solution should be discarded once the nuclei become brown.
If a staining machine is used, follow the instructions of the equipment and software manufacturer.

Sample preparation
All samples must be treated using state-of-the-art technology.
All samples must be clearly labeled.
Suitable instruments must be used for taking samples and for their preparation. Follow the manufacturer's instructions for application/use.

Diagnostics
Diagnoses are only to be made by authorized and trained persons.
Valid nomenclatures must be used.
Suitable controls should be conducted with each application in order to avoid an incorrect result. Further tests must be selected and implemented according to recognized methods.

Storage
Store the dye at +5°C to +30°C.
The dye must be used by the expiry date stated.

Shelf-life
After first opening, the bottle can be used up to the expiry date when stored at +5°C to +30°C.
The bottles must be kept tightly closed at all times.

Instructions for use
For professional use only
In order to avoid errors, the staining process must be carried out by qualified personnel.
National guidelines for work safety and quality assurance must be followed.
Microscopes equipped according to the standard must be used.

Protection against infection
Effective measures must be taken to protect against infection in line with laboratory guidelines.

Instructions for disposal
Used solutions and solutions that are past their shelf-life must be disposed of as special waste in accordance with local guidelines. If you have more detailed questions regarding disposal, these can be forwarded by e-mail: retrologistik@merck.de . Within the EU the currently applicable regulations on the basis of Directive 67/548/EEC of the Council for harmonization of the legal and administrative regulations apply for the classification, packaging, and labelling of hazardous substances.

Auxiliary reagents
Cat. No. 104699	Immersion oil	100 ml, 500 ml
Cat. No. 115577	Immersion oil acc. to ISO 8036 modified	100 ml
Cat. No. 107961	Entellan® new	500 ml
Cat. No. 109016	Neo-Mount®	500 ml
Cat. No. 109843	Neo-Clear®	5 l
Cat. No. 100974	Ethanol denatured with 1% methyl ethyl ketone for analysis EMSURE®	1l, 2.5l
Cat. No. 100983	Ethanol absolute for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1l, 2.5 l
Cat. No. 108681	Xylene for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1l, 2.5l

Hazard classification
Cat. No. 115941
Please observe the hazard classification on the label and the information given in the safety data sheet.
The Merck safety data sheet is available on the Internet and on request.

Main product components
Cat. No. 115941
C.I. No. 42095
$C_{37}H_{34}N_2Na_2O_9S_3$
M = 792.86 g/mol

Status: February 2011

Merck KGaA, 64271 Darmstadt, Germany,
Tel. +49(0)6151 72-2440
www.merck-chemicals.com/microscopy